



Application No. 10/532,423

AMENDMENTS TO THE SPECIFICATION

IN THE SPECIFICATION

On page 29, line 36, please replace the original paragraph with the following amended paragraph:

-- After the spore was formed, conidiophore was scraped away by means of a platinum needle and suspended in 0.01 (v/v)% Tween 80. The suspension was diluted, spreaded onto Czapek-Dox agar medium and cultured at 30°C. Monospore was separated by repeating the above procedures. The separation of the monospore was confirmed by a modified Hondel method (Spore PCR). The conidium was inoculated in a 1.5 ml micro tube containing 200 µl of YPD culture medium by means of the platinum needle and cultured for 40 hours at 30°C. The cultured fungus was transferred into another 1.5 ml micro tube, suspended with 50 µl of a solution for preparing protoplasts (0.8 M KCl, 10 mM citric acid, pH6.5, 2.5mg/ml Lysing enzyme (Sigma Chemical Co.), 2.5 mg/ml Yatalase (TaKaRa)), allowed to stand for one hour at 37°C, heated for 3 min at 95°C, and then allowed to stand for 5 min or more on ice to precipitate the fungus. PCR was done using a 5 ml of the resulting supernatant as a template, and the following synthesized primers:

5'-ATTCGCGAAAATGGTAGCTCGAGGA-3' (SEQ ID NO: 8) and

5'-GTAGAATCACGAATGAGACCTTTGACGACC-3' (SEQ ID NO: 9) for pNG-enoP-hyp;

and

5'-GTAGAATCACGAATGAGACCTTTGACGACC-3' (SEQ ID NO: 10) and

5'-GTTAGTCGACTGACCAATTCCGCAG-3' (SEQ ID NO: 11) for pNG-enoP.--

On page 34, line 32, please replace the original paragraph with the following amended paragraph:

-- An oligonucleotide of 30 base pairs:

5'-GCACAAGGACTGTTTGAACAAGCTGTTTCC-3') (SEQ ID NO: 12), and

an oligonucleotide of 30 base pairs:

(5'-CCAGGCAGACAAGATCTCCCACGGCGCAAT-3') (SEQ ID NO: 13)

were synthesized on the basis of the above internal amino acid sequence and the base sequence of known cutinase, respectively. PCR was carried out using the genomic DNA (100 ng) as a template, Ex taq polymerase (TAKARA SHUZO CO., LTD.) and the resulting pair of primer set with TaKaRa PCR Thermal Cycler PERSONAL (TAKARA SHUZO CO., LTD.) to amplify a probe for southern hybridization. The amplification was done denaturing the template DNA for 3 min at 95°C, and repeating 30 cycles of 95°C for 1 min, 60°C for 1 min, 72°C for 30 seconds, and 72°C for 1 min for a complete extension, followed by being kept at 4°C. Agarose gel electrophoresis of the resulting PCR amplified fragments revealed amplification of a PCR fragment having 300 base pairs.--

On page 39, line 22, please replace the original paragraph with the following amended paragraph:

-- After the spore was formed, conidiophore was scraped away by means of a platinum needle and suspended in 0.01 (v/v%) Tween 80. The suspension was appropriately diluted, poured onto Czapek-Dox agar medium and cultured at 30°C. Monospore was separated repeating the above procedures. The separation of the monospore was confirmed by a modified Hondel method (Spore PCR method: van, Zeiji, C.M., et al. (1997) J. Biotechnol., Jan 3; 59 (3), 221-224) and as described more in detail in the Example 2 (2-2) of the present specification. The following two oligomers were synthesized as the primers for spore PCR:

5'-TGCAGTGGCGGATCCGGTGGAC-3' (SEQ ID NO: 14), and

5'-GTAGAATCACGAATGGAGCCTTTGACGACC-3'(SEQ ID NO: 15).--

On page 45, line 32, please replace the original paragraph with the following amended paragraph:

-- The following PCR primers for the spore of pPTR-gla-hyp were synthesized:

5'-ATTCGCGAAAATGGTAGCTCGAGGA-3'(SEQ ID NO: 16) and

5'-CTGTGTCCCGTATGTAACGGTG -3' (SEQ ID NO: 17)--

On page 48, line 31, please replace the original paragraph with the following amended paragraph:

-- The following PCR primers for spore of pPTR-enoP-hyp were synthesized:

5'-ATTCGCGAAAATGGTAGCTCGAGGA-3' (SEQ ID NO: 18) , and

5'-CTGTGTCCCGTATGTAACGGTG -3' (SEQ ID NO: 19).

The following PCR primers for the spore of pNG-amy were synthesized:

5'-GGTTCGCTTCGTAAGTCTTCCTT-3' (SEQ ID NO: 20), and

5'-GTAGAATCACGAATGAGACCTTTGACGACC-3' (SEQ ID NO: 21)--

On page 51, line 13, please replace the original paragraph with the following amended paragraph:

--(10-2) Cloning of the hydrophobin homologue gene of *A. oryzae* and preparation of a strain highly expressing it

(10-2-1) Cloning of hyp B

Based on the information about an ORF (Open Reading Frame) of hyp B deduced from the BLAST network service, the following two primers for cloning were synthesized so that the above ORF should be contained in an amplified fragment:

5'-CAACCCAACCGTCGACATGAAGTTCT-3' (SEQ ID NO: 22), and

5'-GCCAAATGGCGTCTAGATTACAGACC-3' (SEQ ID NO: 23).--

On page 53, line 3, please replace the original paragraph with the following amended paragraph:

-- The following PCR primers were synthesized for the spore of pNG-gla-hyp B:

5'-CAACCCAACCGTCGACATGAAGTTCT-3' (SEQ ID NO: 24), and

5'-GTAGAATCACGAATGAGACCTTTGACGACC -3' (SEQ ID NO: 25).--

On page 53, line 17, please replace the original paragraph with the following amended paragraph:

--(10-2-4) Cloning of hydrophobin-315

Based on the information about an ORF (Open Reading Frame) of hydrophobin-315 deduced from the BLAST network service, the following two primers for cloning were synthesized so that the above ORF should be contained in an amplified fragment:

5'-CTGCTTCCTTTGTCGACATGAAGGT-3' (SEQ ID NO: 26) and

5'- TCAATGGTCTAGAAGCCCTTGGC-3' (SEQ ID NO: 27)--

On page 54, line 35, please replace the original paragraph with the following amended paragraph:

-- The following PCR primers were synthesized for the spore of pNG-gla- hydrophobin-315:
5'- CTGCTTCCTTTGTCGACATGAAGGT-3' (SEQ ID NO: 28) and
5'-GTAGAATCACGAATGAGACCTTTGACGACC -3' (SEQ ID NO: 29).--

On page 59, line 28, please replace the original paragraph with the following amended paragraph:

-- The following two mix oligonucleotides having 18 bases and 15 bases, respectively, were synthesized based on the comparison of homology between the PBS-degrading enzyme (cutinase) identified in Example 3 and known analogous enzymes derived from *Aspergillus fumigatus*:
{5'-GT(T/C/A/G)GC(T/C/A/G)TG(T/C)CA(A/G)GG(T/C/A/G)GT(T/C/A/G)-3'} (SEQ ID NO: 30) and,
{5'-(G/A)TA(C/T/G/A)CC(C/T/G/A)CC(C/T/G/A)GC(C/T/G/A)AC(T/G/A)AT-3'} (SEQ ID NO: 31).--

On page 61, line 18, please replace the original paragraph with the following amended paragraph:

--(12-2)

PCR was done using the cDNA library comprising the genes of the PBS-degrading enzyme of Example 3, and the above two PBS-degrading enzyme analogues as a template in order to prepare PCR fragments to be inserted into a plasmid for the expression in *E. coli* (pET-12b: Novagen). The following oligomer primer sets were used:

5'-TGCAGTGGCGGATCCGGTGGAC-3' (SEQ ID NO: 32) , and

5'-GACCGGATGGATCCCGAAAATTATCC-3' (SEQ ID NO: 33) for the PBS-degrading enzyme;

5'-GGCAGCAGGGGATCCCATCGCTG-3' (SEQ ID NO: 34), and

5'-CGTAGCCCACACTCGGATCCTAAGCTGAC-3' (SEQ ID NO: 35) for the gene of PBS-degrading enzyme analogue having the open reading frame of 2,713 base pairs; and

5'-GGCGGCTGCGGATCCAGTAGATATC-3' (SEQ ID NO: 36), and

5'-CAGTTCAGGGGGATCCTATAGAGTCC-3' (SEQ ID NO: 37) for the gene of PBS-degrading enzyme analogue having the open reading frame of 1,801 base pairs.--

On page 66, line 30, please replace the original paragraph with the following amended paragraph:

-- The existence of a protein with about 14 kDa was confirmed at around 0 % ammonium sulfate (Fig.20). Since this protein was eluted from the above hydrophobic column at confirmed at around 0 % ammonium sulfate saturation, it seemed to be a highly hydrophobic protein. Amino acid sequence at N-end was determined to be "DASAVLADFNTLST." (SEQ ID NO: 38)--

On page 67, line 18, please replace the original paragraph with the following amended paragraph:

-- The following two sets of oligonucleotides were synthesized with reference to the homologous sequence of *Aspergillus fumigatus* and a codon usage of *Aspergillus oryzae* (<http://www.kazusa.or.jp/codon/>) :

5'-ATGCTCGCCAAGCACGTC-3' (SEQ ID NO: 39) and 5'-GGCCTTCTTGTACTCGGC-3' (SEQ ID NO: 40), and
5'-GACGCAATCTCCACCAC-3' (SEQ ID NO: 41) and 5'-TCAAACGCATCCGCAATCTG-3' (SEQ ID NO: 42).--

On page 69, line 16, please replace the original paragraph with the following amended paragraph:

-- PCR was carried out using the genomic DNA of *Aspergillus oryzae* RIB40 strain as a template and the following pair of oligonucleotides designed based on the base sequences around the desired gene cloned above:

5'-CTTGCATTCAAGTCGACCTGAACAC-3' (SEQ ID NO: 43) and,
5'-CTATTGAACTATGCTTCTAGAAGGCCTAATC-3' (SEQ ID NO: 44).--

On page 70, line 16, please replace the original paragraph with the following amended paragraph:

-- The following PCR primers for spore were synthesized:
5'- CTTGCATTCAAGTCGACCTGAACAC -3' (SEQ ID NO: 45), and

Application No. 10/532,423

5'-GTAGAATCACGAATGGAGCCTTTGACGACC-3' (SEQ ID NO: 46).--



Application No. 10/532,423

AMENDMENTS TO THE SEQUENCE LISTING

IN THE SEQUENCE LISTING

Please replace the Sequence Listing of record with the Substitute Sequence Listing enclosed herewith. The Substitute Sequence Listing in no way introduces new matter into the specification.